YALE UNIVERSITY LIBRARY
3 9002 06712 6566

PRECIPITIN REACTIONS WITH CANCER ANTISERUM

IRVING NORMAN WOLFSON

1942

MUDD
LIBRARY
Medical

General Medical
Library
T113
+Y12
1485

YALE
MEDICAL LIBRARY

GENERAL MEDICAL
LIBRARY

Digitized by the Internet Archive
in 2017 with funding from
Arcadia Fund

https://archive.org/details/precipitinreacti00wolf

PRECIPITIN REACTIONS WITH CANCER ANTISERUM

by

Irving Norman Wolfson, B.A.
Columbia University, 1938

A Thesis
Presented to the Faculty
of the Yale University School of Medicine
in Candidacy for the Degree of
Doctor of Medicine

Department of Surgery
Yale University School of Medicine

1942

I wish to express my appreciation to Dr. Kenneth W. Thompson for his encouragement and advice during the course of this work.

Introduction

With the profound advances offered the field of infectious diseases by advances in immunology, and with the later knowledge that immunologic phenomena are not restricted to bacterial substances, it is not surprising that workers in neoplastic diseases should devote much time and energy to the application of these phenomena to cancer. However, despite an enormous amount of work, there is as yet no unanimous agreement on the fundamental question, "Do specific cancer antibodies exist?" Thus, one experienced worker claims (30), "There is no antibody whose existence is more definitely proved than those of these antimalignant-cell antibodies," while another writer asserts (51) "The demonstration of specific anti-cancer cell substances in the blood of cancer immune animals is not generally accepted." In view of the complicated antigenic pattern of neoplastic tissue, such disagreement is not surprising.

The preparation of malignant cells' antibodies according to Mann and Welker (34) is hampered by the presence of blood in the tumor antigen. This would cause the production of antibodies to the blood proteins as well as to the malignant cells. By adsorbing the antigen on to alumina and injecting the suspension intramuscularly into rabbits these workers prepared antisera in which the blood protein antibody became undetectable, while malignant cells' antibody remained. The experiments described herein were performed to confirm, and, if possible,extend this work.

Among the workers who have obtained evidence for the existence of carcinoma antibodies is Lumsden, who, in 1925 (29), showed that repeated injection into rats of mouse tumor tissue produced antisera which prevented <u>in vitro</u> growth of tumor cells, but not that of other tissue cells.

Later, he and his associates (32) immunized rats against Jensen rat sarcoma and found their sera toxic to malignant cells of any variety, while harmless to normal tissue. Hirszfeld and Halber (20) found that rabbits injected with alcoholic extracts of cancer tissue produced antibodies which reacted with more than half of all tumors tested, while these antibodies failed to react with normal tissues. Morelli (39) injected animals with thyroid cancer, both primary and metastatic to the abdomen, and found that the sera of more than half the animals reacted with the alcoholic extract of homologous tumor. In one case, the serum reacted with extract of mammary carcinoma. Fuchs (12) found that after intravenous injection of a cell free tumor antigen, normal persons develop an antibody, common to all malignant neoplasms, which gradually disappears from the blood in a few months. Attempting to break down the antigenic pattern of malignant tissue into more specific groups, Lehmann-Facius (27) found that the phosphatid fraction of carcinoma extract could be separated into two parts, one of which was strongly separated by specific carcinoma phosphatid antiserum, and the other by fatty acid antiserum. On the basis of his work, Aron (5) concluded that alcoholic extract of urine of cancer patients contained an antigenic principle which gave a precipitin reaction when mixed in suitable proportions with sera from cancer patients. Trawinski (54) injected saline extracts of fresh tumor tissue into rabbits intravenously and produced organ and tumor specific precipitins reacting with the antigen, while the sera of rabbits immunized with normal tissue did not react with the malignant antigen. Working with well standardized animal tumors, Hoyle (23) found that the alcoholic extracts of several types gave positive complement-fixation

tests with the sera of animals bearing these tumors. The antigen appeared to be lipoidal, and was thought to be common for the different tumors. Furthermore, the many serological tests for malignant disease, based upon antigen-antibody reactions (discussed below), are suggestive enough to afford additional evidence for the existence of these substances.

On the other hand, other experiments have failed to reveal specific malignant cells' antibodies, but indicated the antibody was common to all tissues of the particular animal species, and was non-specific for tissue. Thus Pybus and Whitehead (44) found that the sera of rabbits immunized against mouse carcinoma had a harmful effect in vitro, not only upon carcinoma cells, but also upon mouse heart and kidney cells. They also found that the serum of a rabbit that had been injected with human carcinoma supported the growth of carcinomatous cells from mice, although not as well as did normal rabbit serum. The growth of normal mouse tissue was also inhibited by the same antiserum. Morelli (38, 40) and Witebsky and Morelli (56) were unable to confirm a claim (Lehmann-Facius (27)) for the separation of a specific antigen for tumor. However, they did find that antisera produced by the injection of suspensions of "bone sarcoma" reacted with human extracts of sarcoma and carcinoma, and less positively with normal tissues. After adsorption with normal extracts, only tumor antibodies remained in the serum; all antibodies were gone after subsequent adsorption with tumor extracts. Phelps (43) found that both sarcoma cells and normal spleen cells were killed by antiserum to the Jensen rat sarcoma; spleen antiserum behaved the same way. He concluded that there was no specific cancer antiserum. Hirzfeld and Halber (21) produced complement-fixing and precipitin antibodies by injecting rabbits

intravenously with extracts of tuberculous casecus tissue, purulent material, necrotic tissue from infarcts, and malignant tissue. The antibodies reacted with all the antigens, and it was suggested that tumor antibodies are the result of necrotic material present in most malignant tissues, rather than of any malignant antigen per se. Dmachowski (8) obtained rat antiserum which reacted (complement-fixation) with heated tumor antigen, and also, although to a lesser degree, with extracts of normal rat tissue. Sera of rabbits immunized against extract of normal rat muscles were found to contain antibodies which fixed complement with rat tumor even more strongly than with the original antigen. D'Alessandro et al (4) are also cited as obtaining reactions between antiserum to the Jensen sarcoma and extracts of normal organs. Woodhouse (58) came to the conclusion that there is no proof of the existence of specific carcinoma antigen.

Most of the disagreement of the two sets of investigations is apparently due to the presence of normal tissue antibodies in sera of animals injected with extracts of malignant tissue. It would seem likely that some normal tissue inevitably is mixed with the tumor antigen, the separation of pure malignant tissue from normal tissue being difficult. Evidence has accumulated indicating that the species antibodies, produced by concomitant injections of contaminating normal tissue mixed with the tumor antigen, may be separated from the malignant cells' antibodies, and are not formed in response to pure malignant antigen.

Lumsden (31) found that the antibodies which were toxic to cancer cells were present in the euglobulin fraction of the antiserum, while the species antibodies were contained in the pseudoglobulin. Witebsky

and Poplau (57) concluded that suspensions of saline extracts of carcinoma contained three separate antigens - carcinoma, blood, and non-specific. They found that while carcinoma antiserum may react with normal organ extracts, this may be inhibited by lecithin, while the reactivity with malignant tissue extracts remained unchanged. The antibody adsorption experiments of Morelli, and Witebsky and Morelli, cited above, also indicate the separate identities of the species and malignant antibodies. Friedwald and Kidd (9), using complement-fixation tests, found that the sera of rabbits carrying V_2 carcinoma reacted with normal tissue extracts, but reacted much more strongly with extracts of V_2 carcinoma. Furthermore, they showed that heat abolishes the reaction with the normal tissue extracts, as well as with heterologous tumor antigens, while the reaction with the homologous tumor remained unchanged. This antibody was distinguishable from the antibody to the papilloma virus.

Mann and Welker (34) suggested that contaminating species antibodies were due to the presence of blood mixed with the tumor antigen. They succeeded in preparing antisera free from contaminating species antibodies by adsorbing the tumor antigen on alumina and injecting the suspension intramuscularly into rabbits. With this method, the antibodies formed in response to the contaminating blood antigens disappeared in one to six months leaving only tumor antibodies. This was probably due to the fact that blood antigen was present in much smaller quantities than the tumor, and was rapidly exhausted from the intramuscular depots of the alumina mixture, while appreciable amounts of malignant antigen remained. As a result, blood protein antibody production ceased sooner, thereby permitting these antibodies to disappear while only tumor antibodies remained in force.

These latter antibodies were demonstrated by precipitin reactions with an autolyzed antigenic extract; no reaction was obtained with blood protein after one to six months and normal autolyzed tissue extracts did not react either with the antisera. In addition, the antisera reacted with a significant number of sera from cancer patients, and not with sera of control cases, thus furnishing a basis for a useful diagnostic test.

The use of alumina as an adsorbent had previously been described by Marshall and Welker (35) in 1913. Jacobs (24) and Seibert (46) demonstrated that the precipitinogenic power of certain antigens was markedly increased after adsorption with alumina. In 1933, Hektoen and Welker (16) caused the satisfactory formation of precipitins in the rabbit after one intramuscular injection of antigen adsorbed on alumina. In 1934, the same authors (17) showed that when several antigens were adsorbed on alumina, the antibodies formed disappeared from the circulating blood at different times, apparently in proportion to the amount of respective antigen injected. Spinka and Weichselbaum (52) applied this technique to tissue antigens. They immunized rabbits with ground kidney and liver adsorbed on alumina and found that precipitins for blood proteins disappeared in one to two months, at which time the antisera continued to react with autolysates of the antigenic extract. The work of Mann and Welker appears to be an extension of this, using malignant tissue as an antigen.

Material and Methods

Briefly stated, the methods were as follows: an extract, prepared from metastatic thyroid carcinoma, was adsorbed on alumina and injected intramuscularly into four rabbits. Their venous blood at intervals of 3-4 weeks was tested with normal human serum for the presence of blood antibodies. When these had disappeared, the antiserum was then tested with autolyzed extract of the original antigen to determine the presence of antibodies to this preparation. The sera of the rabbits that were shown to be free of blood antibodies and to contain antibodies to the autolyzed extract (presumably tumor cell) were then tested with the sera of patients with and without malignant disease.

Preparation of Antigen:

(1) Tissue. Great difficulty was encountered in procuring a sufficient amount of comparatively "pure culture" of malignant tissue; that is, tissue that is essentially free of normal tissue, without infection, and with a minimum of necrosis. The amount of such material obtainable from surgical specimens was negligible. It was finally possible to obtain satisfactory antigen from the autopsy material provided by a patient with carcinoma of the thyroid with extensive generalized carcinomatosis. The liver of this patient was heavily infiltrated with metastases, and from one slice sixty grams of almost pure malignant tissue were obtained after careful dissection (see Appendix). Lundy (33) has reported that metastatic and autopsy material furnished good antigens for complement-fixation reactions, and Morelli (39) had, in fact, used abdominal metastases from thyroid carcinoma to produce satisfactory antibodies in the rabbit.

After thoroughly dissecting away all normal tissue, the tumor tissue was ground in a meat grinder and washed with saline to remove as much blood

as possible. It was then ground in a ball mill with some saline and a small amount of toluol as a preservative for about sixty hours and strained through a cheesecloth. The filtrate was washed with saline in a centrifuge until the supernatant was biuret negative. The ground tissue was taken up in saline, a few drops of merthiolate, 1:1000, was added, and then it was treated with enough alumina to precipitate all the suspension.

(2) Alumina: The alumina was prepared by slowly adding 1% ammonium hydroxide to a liter of 1% ammonium alum, at room temperature, until the liquid yielded a faint odor of ammonia. The resulting precipitate was washed several times by decantation with distilled water, filtered, and washed until the filtrate was fairly free from salts. It was then transferred to a glass cylinder and washed again by decantation until 500 cc. of the supernatant, evaporated to dryness, left practically no residue.

After the tumor extract had been adsorbed on to the alumina, this was taken up with as much saline as was convenient to permit the use of a 17 gauge needle, and injected into the thigh muscles of four rabbits, twenty to thirty cubic centimeters into each rabbit, in depots of one to two cubic centimeters.

A portion of the tumor extract was not precipitated with alumina, but set aside for tests against the rabbit antisera. This was autolyzed by suspending in three times its volume of saline and incubating for twenty-four hours at 38°C. The resulting suspension was then diluted with saline up to 1:1000.

Technique of Tests: The precipitin tests were carried out in tubes made by sealing off 7 mm. bore glass tubing. Two tenths of a cubic centimeter

of each serum were all that were required for each test, one serum being slowly layered over the other. The tubes were left at room temperature for an hour, and a positive reaction determined by the presence of a thin annular precipitate at the interface between the two sera.

Preliminary experiments were necessary in order to perfect the technique, as it is not difficult to obtain both false negatives and false positives by faulty technique. Factors leading to a false positive reaction include, first of all, dirty tubes and pipettes. A cloudy emulsion easily forms on the glassware, and this may be washed down along the sides by the test serum, settle at the interface, and give a false positive. Although the tubes were left in alkali overnight, rinsed with cleaning solution, and thoroughly washed with water under suction, it was found advisable not to use the same tube more than three times at most. Another source of error lies in the fact that although all sera from patients or rabbits were drawn in the fasting state, they were occasionally cloudy. The appearance in the precipitin tube of the sudden transition from the cloudy lower layer to clear upper layer almost resembled a positive reaction, and frequent controls with saline were necessary as a check. Occasionally droplets of oil would collect just above the interface; these may be distinguished from a true positive because they do not assume an annular configuration, and appear above rather than at the interface.

False negative readings are avoided by reading the tubes with a strong light shining through, preferably against a dark background. Day light, or light of ceiling fixtures, are not sufficiently powerful; a desk lamp produces a satisfactory amount of illumination, and this was

used.

The experimental readings (rabbit antiserum with normal human serum or with antigen) were done in duplicate, and in every case consistent results were obtained. The sera for the clinical readings (rabbit antiserum against patient's sera) were obtained from the sera sent to the surgical chemistry laboratory for analyses. These had all been withdrawn under oil in the fasting state. In this way, the diagnoses of the patients were unknown to the experimentor, and were learned only after the reading had been recorded. These determinations were not done in duplicate because of the limitation in the amount of serum.

Results

The sera of two of the rabbits injected were tested with normal human serum for the presence of antibodies of human blood protein. These tests were performed by layering the human serum, in dilutions of 1:10 - 1:1000, over the rabbit antisera as described above. The data in Table I demonstrate that precipitins to normal blood proteins disappeared from the sera of both rabbits about three months after injection of the antigenic preparation. This interval was found to be one to two months by Spinka and Wechselbaum (52), and one to six months by Mann and Welker (34). The remaining two rabbits died of sepsis a few days after injection (revealed by autopsy).

Having demonstrated the disappearance of contaminating blood antibodies, the antiserum next was tested with the original autolyzed extract to detect the presence of antibodies for this preparation. Of the two rabbits sera tested one gave a positive precipitin reaction with the extract, while the other did not. Simultaneous controls of normal human

serum, layered over undiluted human serum, to check on error in technique, were negative, as were reactions of diluted antigen layered over normal human and normal rabbit sera (Tables I and II).

It may be mentioned that the literature reveals that not many rabbits respond with antibody production to the injection of malignant tissue. Mann and Welker state, "The percentage of rabbits producing powerful precipitin antiserum when immunized with malignant tissue is much smaller than the percentage that responds when immunized with normal serum proteins". Using thyroid tumor, Morelli (39) obtained antibodies in four of seven rabbits, and likewise Hirszfeld and Halber (20) found antibodies in only 10-20% of rabbits injected with alcoholic extracts of cancer tissue.

Following the apparent demonstration of precipitins to malignant tissue in Rabbit #1, the antiserum of this rabbit was then tested with those of patients. In the first tests the antiserum was diluted 1:10; however, in the course of the experiments, the rabbit died* and it was necessary to dilute to 1:50 its remaining serum so that a few additional tests could be performed. It was found that although 1:10 was more satisfactory, positive results were obtainable with the more dilute antiserum.

Five of 10 patients with carcinoma proved by biopsy in almost all cases reacted positively with the antiserum. A total of 13 tests were performed, with 7 positive results, three patients being tested with both dilutions of antiserum. Four of 7 tests with the 1:10 dilution and 3 of 6 with the 1:50 dilution were positive (Table III).

One patient with a benign tumor (meningocytic meningioma) also gave

*Autopsy revealed a fibrinous gray exudate over the epicardium, some cloudy white fluid in the pericardial cavity, a flabby myocardium, and a large, dilated stomach.

TABLE I

Disappearance of Normal Human Blood Protein Precipitins from Two Rabbits Injected with Antigen (Metastatic Thyroid Carcinoma Adsorbed on Alumina).

A. Rabbits No. 1 and 2 — Injected 11-13-41

		Normal human serum		
		1:10	1:100	1:1000
12-21-41	Undiluted antiserum	+	+	+
1-24-42	Undiluted antiserum	+	+	+
2-12-42	Undiluted antiserum	-	-	-
B. Controls				
1-24-42	Undiluted normal	-	-	-
2-12-42	human serum	-	-	-

TABLE II

Positive Precipitin Reaction in One of Two Rabbits Following Disappearance of Human Blood Protein Precipitins.

	Autolyzed Antigenic Suspension		
	1:10*	1:100	1:1000
Rabbit No. 1 (undiluted)	?	+	-
Rabbit No. 2 "	?	-	-
Normal Rabbit "		-	-
Normal Human "		-	-

N.B. All reactions in above tables were performed in duplicate.

*The suspension was too thick at this dilution for a satisfactory reading to be made.

TABLE III

Precipitin Reactions of Sera of Carcinomatous Patients with Rabbit Antiserum or with Carcinoma Extract.

Case No.	Patient	Age	Sex	Hosp. No.	Diagnosis	Metast.	Biopsy	Reaction with: Antiserum 1:10	Antiserum 1:50	Extract 1:100
1	A. A.	70	F	B24755	Ca sigmoid	-	+	-		-
2	A. C.	86	M	A69326	Ca sigmoid	-	+	-		-
3	M. T.	55	F	B24357	Ca rectum	-	+	-		-
4	I. Z.	47	F	26930	Ca rectum	-	+	+		
5	R. A.	64	M	B24853	Ca bronchus	-	+	+	+	-
6	W. H.	80	M	B1392	Ca prostate	?	+	+	+	-
7	L. D.	61	F	B993	Ca stomach	+	+	+	-	-
8	C. A.	66	M	B24713	Ca rectum post-op	-	+		-	-
9	E. K.	43	M	A31100	Ca stomach post-op	+	+		+	-
10	B. W.	57	F	B24587	Ca colon	-	*		-	-
	Patient with Benign Tumor									
	J. M.	56	M	B8981	Meningocytic meningioma	-	+	+		

*Biopsy not reported, but gross appearance at operation was that of unresectable carcinoma.

a positive reaction. This patient had had a gastrectomy about 2 years earlier for a presumed gastric carcinoma, which, however, on pathological study proved to be a benign ulcer.

In all 10 patients without evidence of malignant disease were tested, and they were negative; 7 with 1:10 dilution of the antiserum, and 4 with 1:50 dilution (one patient was tested with both dilutions). These results appear in Table IV.

Because some workers had reported positive antibody-antigen reactions between tumor extracts and the sera of cancer patients, the autolyzed extract was tested against the sera of 9 patients with malignant disease, by layering over these the 1:100 dilution of antigen. All of these were negative (Table III).

Discussion

The amount of data available from the present experiments are hardly sufficient to be considered as a confirmation of the work of Mann and Welker although the results tend to lean that way. The few positive reactions which were obtained with the human sera were all in tumor patients, and the results thus appear to justify further work.

Assuming slight statistical significance to the results, the simplest explanation would be that upon which the theory of the experiment is based - the injection of the alumina-precipitated malignant tissue resulted in the production of precipitin antibodies in the rabbit, which cause the positive reaction with the original antigenic suspension. The reaction between the serum of carcinoma patients and the rabbit antiserum would necessitate the assumption of the circulation of malignant antigen in the patient's blood. Some evidence for this may be found in the work

TABLE IV

Reactions of Sera of Non-Carcinomatous Patients with Rabbit Antiserum

Case No.	Patient	Age	Sex	Hosp. No.	Diagnosis	React. with antiserum 1:10	1:50
1	A. D.	72	M	B24307	Duodenal ulcer	-	
2	H. M.	65	M	B24336R	Duodenal ulcer	-	
3	C. C.	45	F	A29221	Pulmonary tbc. Small bowel obst. due to post-op adhesions	-	
4	E. M.	41	F	A23010	Cystitis Chronic P. I. D.	-	
5	E. D.	13	F	A25566	Perforated appendix	-	
6	M. W.	69	M	B24849	Perforated gall bladder	-	
7	I. W.	23	M		Normal	-	
8	C. J.	70	M	B13207	Perforated appendix	-	-
9	J. S.	44	M	67089	Duodenal ulcer		-
10	L. O.	16	F	B15568	Nephrotic stage of glomerulonephritis Pneumococcal peritonitis		-

of Hirszfeld and his associates (22) who found that the serum of rabbits immunized with human tumor extracts reacted with extract of livers of patients with gastric carcinoma. This was interpreted as implying the impregnation of the liver with carcinomatous substances, a sort of "biochemical metastasis". Breinl and his associates are cited as obtaining similar results (6).

Other diagnostic tests based upon this principle have been described. Gruskin (14), using embryonal cells from fetal mammals injected intraperitoneally into the experimental animals, obtained an antiserum which reacted with carcinoma serum. Peretz and Newler (42) used a complement-fixation test with carcinoma serum as antigen and the sera of rabbits immunized with carcinoma extracts as the source for antibody. With this technique, these workers obtained 75% positives with carcinoma patients, and 7.6% false positives. Fairly good results have been obtained with the urine of carcinoma patients as the source of antigen (5), and this implies the presence of carcinoma antigen in the circulating blood. Against these latter results are the theoretical objection of the difficulty of the antigen, presumably of large molecular weight, to pass through the renal filter, as well as the experimental results of Sobotka and Bloch (50) whose work indicates that the only chemical difference between the urine of carcinoma and non-carcinoma patients lies in a higher cholesterol level of the former.

Although the above explanation might be appealing, several serious objections may be raised. The precipitin reactions were carried out with 1:50 dilution of the antiserum. According to Zinsser and Bayne-Jones (3), precipitin reactions as known at present, are positive only in low dilu-

tions of antiserum, with 1:20 mentioned as a limiting figure. This would suggest that all the positive results were due to faulty technique. On the other hand, the paper of Mann and Welker mentioned positive precipitin reactions between antiserum and normal human blood proteins with dilutions of the former as high as 1:100,000.

The possibility that the precipitins in the rabbit serum were anti-bacterial and not anti-malignant must be considered - especially as two rabbits died within a few days after injection with overwhelming infections despite the use of merthiolate. This possibility is always present when malignant, slightly necrotic (see Appendix) tissue is used. However, while the reaction between antigen and antiserum would be explainable, it would be difficult to account for the positive reaction between the antiserum and carcinoma patients' sera in view of the negative results with the controls. More difficult to answer is the work of Hirszfeld and Halber (21) indicating that the antigenic properties of malignant tissue are in common with those of all necrotic tissue. It may be pointed out, however, that several of the negative controls suffered from acute gangrenous processes, which, theoretically, should have caused the liberation of as much "necrotic antigen" as some of the carcinoma patients.

The second series of tests, using the antigenic suspension and patients' antisera, was based upon the possible presence of antibodies to malignant tissue in the patient's blood, rather than circulating malignant antigen. These tests were all negative. However, several diagnostic tests for carcinoma, based upon this supposition, have been devised by others. Some of these have resulted in significant, although not clinically useful, results. The technique has usually been a complement-fixation test, rather

than precipitin, with tumor extract (usually alcoholic, and occasionally cholesterolized) as antigen and the patients' sera as antibody.

In 1908, Simon and Thomas (49) used breast tumor emulsion as antigen for complement-fixation tests and obtained suggestive results (65% positive in 37 malignant cases, and 4% positive in 50 controls). Fry (10, 11) used a saline emulsion of the saline insoluble, acetone and alcohol soluble, fraction of malignant tissue and obtained about 75% correct results. Other workers have obtained satisfactory results with complement-fixation or flocculation tests based upon the above theory (15, 26, 37, 41, 45, 55). Less satisfactory results have been obtained by others (18, 28, 36, 47, 48, 53).

It is evident that these problems are in an unsettled state, and deserve further exploration. The work reported here, though suggestive, is of too small volume to help to clarify the subject.

Summary and Conclusions

1. Four rabbits were injected intramuscularly with human metastatic thyroid carcinoma adsorbed upon alumina. Two rabbits died of infection a few days after their injections.
2. Precipitins to normal human blood serum, demonstrated in the sera of the two surviving rabbits, disappeared three months after the injection.
3. The serum of one of the two rabbits gave a positive precipitin reaction with the autolyzed antigen after the human blood precipitins had disappeared.
4. The serum of this rabbit gave a positive precipitin reaction with the sera of 5 of 10 patients suffering with malignant disease. Of 13 tests

performed on such patients, 7 were positive. The presence or absence of clinically demonstrable metastases did not appear to affect the result.

5. Negative reactions with the antiserum were obtained with serum of all 10 people without demonstrable cancer.
6. The single case with a benign tumor (meningioma) reacted positively.
7. Precipitin reactions between all sera of 9 carcinomatous patients and the autolyzed extract were negative.
8. The small number of experimental animals and tests used in this work prevent any definite conclusions from being drawn.
9. The theoretical aspects and other experimental work in this field are discussed.

Appendix

Excerpts from Autopsy Protocol of R. W., Hosp. No. B15824, Autopsy No. 5548.

Anatomical Diagnosis

"Carcinoma of thyroid with metastases to mediastinal, axillary, cervical, retroperitoneal and inguinal lymph nodes, internal mammary veins, epicardium, lungs, liver, left kidney and vertebrae.

"The thyroid has the usual butterfly shape and weighs 28 grams. External color is much lighter red-brown than usually encountered. When sectioned it is discovered that about 40-50% of the parenchyma is replaced by firm gray, gritty tissue.

".... in all, the normal thyroid acini have been almost completely replaced by neoplastic tissue. The latter is composed of cells with large, oval or round variable-sized vesicular nuclei which occasionally contain nucleoli; cytoplasm is faintly eosinophilic, scant, and faintly granular. Mitotic figures are present but not numerous, there seldom being more than two in each high dry (X450) field. For the most part these cells occur in solid masses or sheets, without distinctive pattern. Not infrequently, however, small well-formed acini are found in the neoplastic tissue. Dense fibrous tissue surrounds all foci of the malignant cells.

"The liver weighs 3900 grams. Its surface is studded with many large and small discrete and confluent umbilicated nodules of gray-white tissue ... In section, the accumulations of firm white tissue are found to occupy an estimated 50-60% of the bulk of the liver. Centrally, the foreign tissue foci are found to be softened with the result that cream-like material can be scraped away on the knife blade.

"Large sheets and masses of tumor cells are scattered about in a fibrous stroma containing proliferating bile ducts ... Several small malignant tissue implants removed from the main large nodules are seen scattered throughout the parenchyma. One small group of neoplastic cells occupies the lumen of a central vein."

BIBLIOGRAPHY

Monographs

1. Landsteiner, K.: The Specificity of Serological Reactions. Springfield, Illinois, Charles C. Thomas. (1936).

2. Wells, H.: The Chemical Aspects of Immunity. New York, N. Y. The Chemical Catalogue Co. (1929).

3. Zinsser, H. and Bayne-Jones, S.: Textbook of Bacteriology. New York, N. Y. D. Appleton-Century Co. (1939).

Articles

4. d'Alessandro, G. and Galgio, L.: Uber die serologische Spezifiziti des Tumorgewebes. Ztschr. f. Immunitat u. exper. Therap. 87: 137 (1936). cit. by Dmachowski (8).

5. Aron, M.: Recherche serologique sur la nature du principe contenu dans l'urine des cancereux. Compt. rend. Acad. de Sci. 203: 1550 (1936).

6. Breinl, F., Chrobok, E. and Kindermann, V.: Beitrag zur Serologie der Tumoren. Ztschr. f. Immunitäts. u. exper. Therap. 86: 260 (1935). cit. by Dmachowski (8).

7. Clemente, G.: Contribution a L'Etude Serologique du Cancer Les Neoplasmes. 5: 294 (1926).

8. Dmachowski, L.: Serological Specificity of Experimental Tumors. Amer. Jour. Canc. 37: 252 (1939).

9. Friedwald, W. F. and Kidd, J. G.: Distinct V_2 Carcinoma Antibodies. Proc. Soc. Exp. Biol. and Med. 47: 130 (1941).

10. Fry, J. B.: Flocculation Reaction for the Serum Diagnosis of Malignant Disease. Jour. Path. and Bact. 29: 353 (1926).

11. Fry, J. B.: A New Flocculation Reaction for the Serum Diagnosis of Malignant Disease. Brit. Med. Jour. 2: 4 (1925).

12. Fuchs, H. J.: Uber Tumorimmunitat Klin. Wchnschr. 13: 292 (1934). Abst.: Amer. Jour. Canc. 21: 683 (1934).

13. Geisthovel, W.: Klinischer Beitrag zur serologischer Krebsdiagnose. Zentralbl. g. Chir. 65: 2642 (1938). Abst.: Amer. Jour. Canc. 39: 122 (1940).

14. Gruskin, B.: Serum Test for Diagnosis of Cancer. Amer. Jour. Med. Sci., 177: 476 (1929).

15. Von Gutfield, F.: Klinische Wertung der Komplementbindungsreaktion mit

akloholischen Krebsextrakten. Klin. Wchnschr. 10: 2308 (1931). Abst.: Amer. Jour. Canc. 16: 1047 (1932).

16. Hektoen, L. and Welker, W. H.: Precipitin Production in Rabbits Following Intra-muscular Injection of Antigen Adsorbed by Aluminumhydroxide. Jour. Inf. Dis. 53: 309 (1933).

17. Hektoen, L. and Welker, W. H.: Precipitinogenic Action of Human Plasma and Its Constituents. Ibid. 55: 271 (1934).

18. Hirasawa, S.: Experimentelle Studien uber die Immunitat der Karzinome. Trans. Soc. path. jap. 25: 704 (1935). Abst.: Amer. Jour. Canc. 29: 280 (1937).

19. Hirszfeld, L. and Halber, W.: Uber komplementbindende Antikranken und Schwangenen. Ztschr. f. Immunitats u. exper. Therap. 67: 286 (1930). Abst.: Amer. Jour. Canc. 15: 373 (1931).

20. Hirszfeld, L. and Halber, W.: Uber Krebsantikörper bei Krebskranken. Klin. Wchnschr. 9: 342 (1930). cit. by Lundy (33).

21. Hirszfeld, L. and Halber, W.: Beiträge zur Immunbiologie des Tuberkulösen Käses, des Eiters und des Krebses. Klin. Wchnschr. 16: 878 (1937). Abst.: Amer. Jour. Canc. 37: 127 (1939).

22. Hirszfeld, L., Halber, W. and Laskowski, J.: Uber die serologische Spezifizitie des Krebszellen. Klin. Wchnschr. 8: 1563 (1929). cit. by Dmachowski, L. (8).

23. Hoyle, L.: A Lipoidal Antigen Produced by Certain Malignant Tumors of the Mouse. 59: 479 (1934).

24. Jacobs, J.: Adsorbents in Immunizations with Haptenes. Jour. Exp. Med., 59: 479 (1934).

25. Klopstock, F. and Simon, E.: Zur Frage des Vorkommens tumorspezifischer Antigene in bosartigen Geschwulsten. Ztschr. f. Krebsfirsch. 37: 127 (1932). Abst.: Amer. Jour. Canc. 16: 1277 (1932).

26. Landau, J. L. and German, W. M.: Serodiagnosis of Malignant Disease. Amer. Jour. of Clin. Path. 2: 343 (1932).

27. Lehmann-Facius, H.: Chemisch-serologische Fraktionerung und Spezifitat von Karzinomatrakten. Ztschr. f. Immunitat u. Exper. Therap. 82: 99 (1934). Abst.: Amer. Jour. Canc. 23: 133 (1935).

28. Lipkin, A. I.: La reazione di fissazione del complimento nel cancro secondo il metodo di Hirschfeld. Gior. di. batteriol. e. immunol. 9: 605 (1932). Abst.: Amer. Jour. Canc. 21: 126 (1934).

29. Lumsden, T.: The Effect of an antiserum upon Cancer Cells in Vitro. Lancet. 1: 383 (1925).

30. Lumsden, T.: Tumour Immunity. Amer. Jour. Canc. 25: 563 (1931).

31. Lumsden, T.: Tumour Immunity. Jour. Path. and Bact. 34: 349 (1931).

32. Lumsden, T., Macrae, T. and Skipper, F.: Direct Demonstration of Anti-Cancer Bodies in Serum of Animals Immune to Homologous Tumour. Jour. Path. and Bact. 39: 595 (1934).

33. Lundy, H. W.: Complement-fixation Reactions in Carcinoma. Jour. Lab. and Clin. Med. 20: 923 (1934-35).

34. Mann, L. S. and Welker, W. H.: A Specific Precipitin Antiserum for Carcinoma Protein. Amer. Jour. Canc. 39: 360 (1940).

35. Marshall, J. and Welker, W. H.: Precipitation of Enzymes from their Solutions by Moist Aluminium Hydroxide. Jour. Amer. Chem. Soc. 35: 822 (1913).

36. Marzynski, J. and Silberstrom, L.: Die Krebsreaktion von Hirszfeld und Halber. Klin. Wchnschr. 11: 290 (1932). cit. by von Gutfield (15).

37. Montpellier, M. J. et al: Premieres recherches sur le serodiagnostic du cancer. L'Algerie Medicale. 37: 266 (1933).

38. Morelli, E.: Ulteriori studii sul metabolismo dei lipoidi rei tumori maligni. Arch. di Biol. 86: 377 (1932). Abst.: Amer. Jour. Canc. 19: 860 (1933).

39. Morelli, E.: Ulteriore contributo allo della natura e della specificita degli antigeni neoplastici. Boll. d. lega. ital. per la lotta contro il cancro. 7: 203 (1933). Abst.: Amer. Jour. Canc. 22: 682 (1934).

40. Morelli, E.: Weiteres uber due Fraktionierung von Geschwulstantigeber. Ztschr. f. Imm. u. exper. Therap. 83: 521 (1934). Abst.: Amer. Jour. Canc. 25: 866 (1935).

41. Munter, H.: Beitrage zur serologischen Krebsdiagnose. Med. Klin. 28: 1312 (1932). Abst.: Amer. Jour. Canc. 18: 160 (1933).

42. Peretz, L. H. and Newler, A. J.: Zur Frage der Serodiagnose des Krebses. Ztschr. f. Krebsforsch. 41: 267 (1934). Abst.: Amer. Jour. Canc. 24: 157 (1935).

43. Phelps, H. J.: A Note on the Non-specific Action of So-called "Anti-cancer Serum". Amer. Jour. Canc. 31: 441 (1937).

44. Pybus, F. C. and Whitehead, H. R.: Immune Reactions and Cancer. Jour. Path. and Bact. 32: 195 (1929).

45. Saphir, W. and Hirschberg, N.: Complement-fixation Test in Carcinoma. Jour. Immunol. 25: 439 (1933).

46. Seibert, F. B.: Composition of the Active Principle of Tuberculin. Jour.

Immunol. 28: 425 (1935).

47. Sievers, O.: Hirszfield Complement-Fixation Reaction and Kahn Albumin A Reaction in Cancer. Finski Läk. Handlingar (Helsinfors). 73: 7 (1931). Abst.: Amer. Jour. Canc. 15: 1661 (1931).

48. Sievers, O.: Komplementbindungsversuche beim Karzinom. Ztschr. f. Immunitat. und exper. Therap. 74: 257 (1932). Abst.: Amer. Jour. Canc. 18: 160 (1933).

49. Simon, C. E. and Thomas, W. S.: On Complement-Fixation in Malignant Disease. Jour. Exp. Med. 10: 673 (1908).

50. Sobotka, H. and Bloch, E.: Urine Extractives in Cancer. Amer. Jour. Canc. 35: 50 (1939).

51. Spencer, R. R.: Tumour Immunity. Jour. Nat. Canc. Inst. 2: 317 (1942).

52. Spinka, I. and Weichselbaum, P. K.: Antisera for Organ Proteins. Proc. Soc. Exp. Biol. and Med. 38: 447 (1939).

53. Tinozzi, F. P.: Il metodo di deviazione del complimento di Hirzfeld. Diag. e. tech. di lab. 3: 6 (1932). Abst.: Amer. Jour. Canc. 16: 784 (1932).

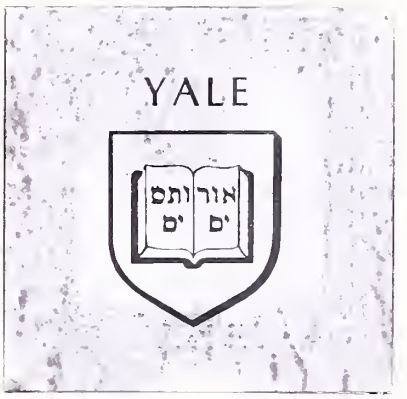
54. Trawinski, A.: Über Nachweis immunobiologischer Prozesse bei Krebs mittels Prazipitationsmethode. Ztschr. f. Immun. 90: 65 (1937). Abst.: Amer. Jour. Canc. 38: 422 (1940).

55. Weiss, E.: A Coagulo flocculation Test for Malignant Tumors. Arch. Path. 13: 106 (1932).

56. Witebsky, E. and Morelli, M.: Zur serologischen Analyse des Sashomgewebes. Ztschr. f. Immunitat. u. exper. Therap. 78: 179 (1933).

57. Witebsky, E. and Poplau, P.: Zur Methodik des Nachweis karzenomspezifischen Antigen funktionem. Ztschr. f. Immunitat. 76: 82 (1932). cit. by Lundy (33).

58. Woodhouse, D. L.: Chemodiagnosis of Malignancy. Amer. Jour. Canc. 40: 359 (1940).


YALE
אור
ותם